HIPPOCAMPAL STRATA THETA OSCILLATIONS CHANGE THEIR FREQUENCY AND COUPLING DURING SPATIAL LEARNING

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Abstract—The theta rhythm is necessary for hippocampal-dependent spatial learning. It has been proposed that each hippocampal stratum can generate a current theta dipole. Therefore, considering that each hippocampal circuit (CA1, CA3, and Dentate Gyrus (DG)) contributes differently to distinct aspects of a spatial memory, the theta oscillations on each stratum and their couplings may exhibit oscillatory dynamics associated with different stages of learning. To test this hypothesis, the theta oscillations from five hippocampal strata were recorded in the rat during different stages of learning in a Morris maze. The peak power, the relative power (RP) and the coherence between hippocampal strata were analyzed. The early acquisition stage of the Morris task was characterized by the predominance of slow frequency theta activity and high coupling between specific hippocampal strata at slow frequencies. However, on the last training day, the theta oscillations were faster in all hippocampal strata, with tighter coupling at fast frequencies between the CA3 pyramidal stratum and other strata. Our results suggest that modifications to the theta frequency and its coupling can be a means by which the hippocampus differentially operates during acquisition and retrieval states. © 2016 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: Morris water maze, electrode array, learning and memory, theta oscillations, navigational learning.

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Abbreviations: ANOVA, analysis of variance; DG, Dentate Gyrus; LFP, local field potential; RP, relative power; SAS, synchronizing ascending system; sm, moleculare stratum; sml, lacunosum-moleculare stratum; spCA1, pyramidal stratum (CA1); spCA3, pyramidal stratum (CA3); sr, radiatum stratum.

INTRODUCTION

The theta rhythm is a predominant pattern in hippocampal circuits during REM sleep and during active behaviors that involve external-world information processing, such as walking, running, jumping, swimming, rearing, head moving, sniffing, and whisking (Vanderwolf, 1969; Winson, 1978; Leung, 1984; Vinogradova, 1995); during periods of immobility with highly aroused states due to a previously conditioned stimuli (Vanderwolf, 1969; Whishaw, 1972; Bland, 1986) or the presence of a predator (Sainsbury et al., 1987); and during time discrimination periods (Nakazono et al., 2015). This activity pattern is present and necessary for hippocampal-dependent spatial learning (Winson, 1978; Mitchell et al., 1982; Mizumori et al., 1990; Turnbull et al., 1994; Buzsáki, 2002; McNaughton et al., 2006b; Shirvalkar et al., 2010). However, the mechanism by which theta activity contributes to hippocampal function is unknown.

It has been suggested that the theta rhythm may synchronize neural populations to enable information transfer within and across neural circuits (Sirota et al., 2008; Gordon, 2011; Buzsáki and Watson, 2012). Consistent with this idea, several studies have shown that the gamma oscillations and firing of neurons in the hippocampus (Bragin et al., 1995; Csicsvari et al., 2003; Colgin et al., 2009; Montgomery et al., 2009) and neocortical areas are phase-locked to the hippocampal theta rhythm (Jones and Wilson, 2005; Siapas et al., 2005; Canolty et al., 2006; Sirota et al., 2008; Colgin et al., 2009). Additionally, it has been found that the oscillatory activity in other circuits, such as the prefrontal cortex, amygdala, striatum, medial septum, and supramammillary nucleus, can be synchronized with hippocampal theta oscillations during cognitive processes (Seidenbecher et al., 2003; DeCoteau et al., 2007; Adhikari et al., 2010; Benchenane et al., 2010; Hernández-Pérez et al., 2015). Therefore, the theta rhythm may participate in the local computational operations in the hippocampal circuits and in the synchronization of widely distributed subcortical and cortical networks in order to construct temporary functional circuits. Moreover, the theta rhythm cannot be considered as a global clock, because the global theta activity recorded in LFP depends on the proportional contribution of multiple theta dipoles found in the different hippocampal strata (Feenstra and Holsheimer, 1979; Buzsáki et al., 1983; Leung, 1984; Montgomery et al., 2009). Additionally, the generation of traveling waves that propagate from the septal to the temporal axis has been

demonstrated (Petsche and Stumpf, 1960; Lubenov and Siapas, 2009; Patel et al., 2012).

The intra-hippocampal circuit has a highly organized connectivity. In this sense, the CA1 area integrates information coming from CA3 and entorhinal cortex areas, whose afferents arrive in the radiatum and lacunosum-molecular strata, respectively (Witter and Amaral, 2004). A characteristic of hippocampal circuitry is that each excitatory input is matched by a specific inhibitory input provided by GABAergic interneurons (Freund and Buzsáki, 1996; Witter and Amaral, 2004; Klausberger and Somogyi, 2008). It is also known that a wide subpopulation of interneurons is phase-locked to the theta rhythm (Somogyi and Klausberger, 2005); therefore, it has been suggested that the interplay of excitatory and inhibitory theta patterns may generate a current dipole at each layer or stratum (Buzsáki, 2002; Montgomery et al., 2009). In this picture, the theta oscillations reflect the activity in the circuits as well as the interaction among themselves. Therefore, the theta oscillations may change as a result of distinct computational operations in the intra-hippocampal circuits during the mnemonic process.

An interesting recent study found stratum-specific variations in theta oscillations during the performance of a memory task (Montgomery et al., 2009); however, that study, like a large amount of other work, focused primarily on the study of the dynamics of hippocampal theta during stage retrieval and/or decision making (Schmidt et al., 2013; Belchior et al., 2014), analyzing the oscillatory patterns during the performance of a memory task that has already been learned. Instead of this process, we are interested in studying the oscillatory dynamics of the theta frequency of intra-hippocampal circuits during navigational learning in order to understand how the oscillators of each stratum are organized during the learning stage. It is proposed that if the theta activity in the hippocampal strata plays a role in the processing of spatial information, it may vary in function of learning from the early to late training phase.

For this purpose, we used the place version of the Morris water maze task, which is a successful test to evaluate hippocampal-dependent navigational learning. In previous work, modifications in hippocampal theta oscillations during the learning of the water maze in the CA1 and Dentate Gyrus (DG) have been shown (Olvera-Cortés et al., 2002, 2004; Gutiérrez-Guzmán et al., 2011, 2012; Hernández-Pérez et al., 2015), but the dynamics of the intra-hippocampal circuits during spatial learning of the Morris maze have not been addressed. Therefore, in the present study, we recorded theta oscillations concurrently in multiple hippocampal strata using an 8-electrode (180-micron spacing) linear array in order to record the oscillations generated in the hippocampal strata: Pyramidal CA1 (spCA1), Radiatum (sr), Lacunosum-Moleculare (sml), Moleculare (sm) and Pyramidal CA3 (spCA3). Because it is known that the CA1, CA3 and DG hippocampal areas differently contribute to distinct aspects of spatial learning and memory (Marr, 1971; Treves and Rolls, 1992; Kesner et al., 2004; Lee and Kesner, 2004; Colgin et al., 2008; Yassa and Stark, 2011; Carr and Frank, 2012), our main objective was to analyze whether stratum-specific modifications in the frequency and/or coherence theta occur that reflect specific oscillatory dynamics associated with different stages of navigational learning.

EXPERIMENTAL PROCEDURES

Subjects

Six Sprague–Dawley male rats (age, 4–5 months; 450–500 g) were used. All experiments were performed in accordance with the National Institute of Health guide for the care and use of laboratory animals (NIH Publications No. 80-23) and with the "Norma Oficial Mexicana para el uso de animales de laboratorio" (NOM-062-ZOO-1999). This work was approved by the Research and Ethics Committee of the Instituto Mexicano del Seguro Social, México. The rats were maintained under standard vivarium conditions with 12 h/12 h light–dark cycles, with the temperature controlled at $22 \pm 2\,°C$, and with food and water available ad libitum. All experiments were carried out during the light period, starting at 10:00 am. The experiment test was carried out in groups of three animals. The order in which to start the behavioral test was counterbalanced in such way that each animal started the training between 10:00 and 11:00 am.

Electrode construction

To record the theta oscillations corresponding to each hippocampal stratum, an electrode array was built with eight nichrome wires (25 μm in diameter) and was mounted inside a guide cannula. The electrodes were aligned and spaced 180 μm apart in order to reach five hippocampal strata: spCA1, sr, sml, sm, and spCA3 (Fig. 1A, B). The electrode array was attached to Triangle Biosystems International© Electrode Interface Boards (EIB), and a Microdrive was built (Vandecasteele et al., 2012) in order to adjust the electrode array in an optimal recording position.

Surgical procedures for the implantation of electrodes

Each rat was anesthetized with a mixed solution of ketamine (60 mg/kg) and xylazine (10 mg/kg) and was fixed in a stereotaxic instrument (AnyAngle, Stoelting). The scalp was disinfected with iodine solution, and local anesthetic (Lidocaine, 0.5 ml of 20 mg/ml) was infiltrated subcutaneously before any surgical incision. The skin was gently retracted from the skull, and on the surface of the exposed skull, craniotomy (1.8 mm in diameter, Trephine bone drill bits, BASi) was performed over the right dorsal hippocampus to implant the electrode array (HPC, 3.8 mm posterior, and 3.5 mm lateral to bregma). For two rats, the electrode array was slowly lowered to 3.0 mm below the brain surface and fixed to the skull with dental acrylic, while for the four remaining rats, the electrode array was slowly lowered to approximately 1.8 mm below the brain surface and carefully fixed to the Microdrive with dental cement. The Microdrive was